Remarks

Applicants and the undersigned reviewed the pending Office Action carefully, before preparing this response. Reconsideration is respectfully requested. Nonetheless, in light of the present positions, this application is believed to be in condition for allowance.

Objections were made to the specification. Applicants appreciate the Examiner's attention to several informalities. Responsive thereto, a substitute specification will follow as a supplement to this response.

Several objections were, likewise, raised with respect to claims 2-4, 12 and 14. Again, Applicants appreciate the Examiner's attention to detail. Responsive thereto, the claims indicated are hereby amended to address the subject informalities.

Several claims were also rejected under 35 U.S.C. § 112, second paragraph, as being indefinite. Again, Applicants appreciate the Examiner's concern. Responsive thereto, several amendments are hereby presented. As such, the rejection should be withdrawn, with the subject claims allowed to proceed toward issue.

Claim 1 was rejected under 35 U.S.C. § 103 as unpatentable over Macklin and Britton, in view of Buck. Again, Applicants appreciate the Examiner's concern, but respectfully disagree. The combination of cited references does not establish *prima facie* obviousness, for reasons of the sort discussed below.

Macklin, et al, provides a composition containing recombinant nucleic acid molecules which encode at least two M. tuberculosis antigens. The composition is used as a reagent in various nucleic acid immunization strategies. Nowhere in the description does Macklin, et al. teach or provide any indication for identification of *Mycobacterium tuberculosis.* The Examiner cites that Macklin et al teach an esat 6 primer sequence that is substantially identical to the sequence of SEQ ID NO: 3. However, the applicant would like to assert the fact in case of nucleotide sequences, change of a single nucleotide, alters the full function and scope of the sequence. Please see the sequence alignment below:

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SEQ ID NO:3      GCG GAT CCC ATG ACA GAG CAG CAG TGG A-- 28
MACKLIN Et al.   GGA GCT AGC ATG ACA GAG CAG CAG TGG AAT 30
                 *   * *   * *** *** *** *** *** *** *
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As clearly seen from the alignment, the two sequences are significantly different.

Britton et al discloses SEQ ID NO: 17, the nucleotide sequence encoding M. tuberculosis antigen esat-6 (Genbank Accession No. AF420491). SEQ ID NO: 17 is a 315 nucleotide long sequence, the primer sequences (SEQ ID NO: 3 and SEQ ID NO: 4) as disclosed in the present invention are expected to match a part of the whole gene sequence of esat-6 gene as disclosed in Britton et al. Also, as cited by the examiner that Britton teaches composition and method for treatment of diseases including infectious diseases. But, the Britton et al., document uses Antigen Antibody based test methods unlike the present invention. Also, it would require a lot of undue experimentation for a person skilled in the art to come to the conclusion that Mycobacterium can be detected and even if such a conclusion is reached, he cannot differentiate between *M. tuberculosis and M. Bovis*.

Further, the applicant would like to bring to the notice of the examiner that the present invention involves a thorough experimentation and analysis and has shown certain unexpected results, which were unexplored by anyone in the prior art. The present disclosure for the first time unravels the primers and the method of differentiating Mycobacterium *tuberculosis from M. bovis.* Even though primers specific for *Mycobacterium tuberculosis* complex (which includes *M. bovis*) have been reported, it is for the first time that primers have been identified which can differentiate *Mycobacterium tuberculosis from M .bovis* based on the ESAT-6 gene sequences.

"...It is important to differentiate *Mycobacterium tuberculosis from M .bovis* as *M. bovis* can be prevalent due to post BCG vaccination infection. In India, BCG (Bacillus of Calmette and Guerin) vaccine is administered in early childhood to all children. The vaccine contains mutated BCG strain of *M. bovis.* So far there are no rapid and direct molecular methods of making exclusive diagnosis of *M. tuberculosis* infection minus *M. bovis*. Moreover, the limitation of antibody detection assays using ESAT-6 antigen is enormous in India as stated earlier. The *Mycobacterium bovis* is also an important cause of tuberculosis in animals (cattle) of India and traditionally the Indian farmers who consume raw cow milk will have antibodies against these bacteria. Therefore the antibody detection methods will give false positive report of tuberculosis in otherwise healthy individuals and many patients may undergo unnecessary anti-tuberculosis

treatment while not treating the actual disease. Therefore, our invention will revolutionize the *M. tuberculosis* species-specific diagnosis by using multiplex PCR (using 16S rRNA and *esat-6* primers) directly on the clinical samples or using single *esat-6* primers on the culture isolates. 1." (Please see Page 15-16 and Table 2 of the present invention).

As disclosed in the specification, figure 2 shows the amplification profile of some of the samples which were positive for the presence of Mycobacterium species based on genus specific 16s rRNA PCR assay and the other conventional method of diagnosis It is clear from Fig. 2 that all the lanes did not show the presence of amplified product of size 320 bp. Lane 16 did not show any amplification and it corresponds to *M. bovis* strain. This clearly indicate that this assay based on ESAT 6 gene is specific for *M.tuberculosis.* Table 2 gives the data of the species specific assay using different strains of *Mycobacterium.* It is clear from Table 2 and Fig. 2 that the PCR assay based on *esat*-6 gene is specific for *M.tuberculosis.*

The present invention shows its utility in species-specific and rapid molecular diagnosis of tuberculosis using *esat*-6 gene amplification, for the first time.

Also, the antigen- antibody based test methods have been a research topic but no antigen has been found to be satisfactory. Most of the genus-specific antigens failed because they cross reacted with the environmental *Mycobacteria* and with BCG which is given as a vaccine. Even the *M. tuberculosis* specific antigen (ESAT-6 antigen in this case) has a fundamental drawback of poor predictive value to make an organ specific disease diagnosis. The detection of antibodies against ESAT-6 protein has been found to be somewhat useful in tuberculosis non-endemic countries, its utility in TB endemic countries such as whole of Asia, Africa, Russia and other South American countries is very limited due to sub-clinical exposures of the population . Obviously all the exposed persons will have circulating antibodies in their blood against this antigen. Therefore, if a pre-exposed asymptomatic person (antibodies already positive) gets fresh TB brain infection (TB meningitis) and in another pre-exposed person there is no such fresh infection, the antibody detection assays will not be useful for the specific diagnostic use in such cases, as both these patients will be positive for these antibodies. To explain it in other words, antibody detection assays, for diseases of high endemicity (e.g. Tuberculosis

which is airborne) have very poor specificity and organ specific diagnosis can not be made at all, as the antibody detection methods are indirect evidences of infection.

On the other hand the molecular methods such as PCR are highly specific as they detect the genome of the living organism which is responsible for the disease. In other words, using PCR amplification, the specific diagnosis of tubercular meningitis, abdominal tuberculosis, gastrointestinal tuberculosis, genitourinary tuberculosis besides the pulmonary tuberculosis can be carried out. Also the PCR amplification will detect only active diseases and not the old exposures. Moreover the biggest advantage of molecular methods is that the DNA of causative agent (Mycobacterium) can be detected from old samples such as mummies, fossils etc. which is not possible using antibodies.

Therefore, even if the ESAT 6 gene sequence is disclosed in Britton et al. and a primer of ESAT 6 gene is disclosed in Macklin et al., none of the two provide a method for identification of *M. tuberculosis* and further differentiate it with *M. bovis*. Moreover, it is not obvious to design a primer, knowing the whole sequence of a gene. Knowing the primer from Macklin et al., does not lead to the conclusion of identifying SEQ ID: 3 and SEQ ID: 4 and both the sequences are quite different from the sequence of Macklin et al. An ordinary person in the prior art cannot be motivated to perform experiments to determine the genus and species specificity of primers of ESAT gene. Further, the examiner cites Buck et al., which provides a general teaching about equivalency of primers. However the applicants emphasize the fact that, without knowing the advantages of ESAT gene a person skilled in the art would not have an incentive to consider the use of ESAT over conventional biochemical methods.

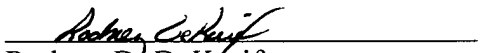
As shown, the primer pair of claim 1 is outside the customary knowledge of one skilled in the art. As such, there is no *prima facie* obviousness. Nonetheless, the surprising and unexpected results observed are quite *uncommon* and serve to show that the present invention is not obvious. Regardless, the rejection should be withdrawn, with claim 1 allowed to proceed toward issue.

Claims 2-11 were rejected under 35 U.S.C. § 103 as unpatentable over Young in view of Macklin and Britton. Again, Applicants appreciate the Examiner's concern, but respectfully disagree. As discussed above, there is no *prima facie* obviousness. The rejection should be withdrawn, with the subject claims allowed to proceed toward issue.

Lastly, claims 12-18 were rejected under 35 U.S.C. § 103 as unpatentable over Young in view of Ecker, in further view of Macklin and Britton. Again, Applicants appreciate the Examiner's concern, but respectfully disagree. Section 103 requires that obviousness be determined on the basis of the claimed subject matter as a whole. Various deficiencies in the cited combination are as discussed above. Further, in addition to the acknowledged deficiencies of Young and Ecker, it appears the Examiner did not consider step (v) of claim 12. No reference, alone or in combination with another, teach or suggest differential indication of *Mycobacterium* species. *Prima facie* obviousness cannot be established on less than the entire claimed subject matter. As such, the rejection should be withdrawn, with the subject claims allowed to proceed toward issue.

This application is now believed to be in condition for allowance. Consistent therewith, favorable action is respectfully requested. The Examiner is invited to contact the undersigned by telephone should any issue remain. Thank you for your help and consideration.

Respectfully submitted:



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